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SCREENING TESTS FOR THE DETECTION OF ABNORMAL MILK

Public Health Service
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1965

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Screening Tests for the Detection of Abnormal Milk

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Springfield VA 22151

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services (Environmental Health)
Division of Environmental Engineering and Food Protection
Washington, D.C. - 20201
1965

Details of illustrations in
this document may be better
studied on microfiche

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

Evaluation check list for Wisconsin Mastitis Test

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ADDENDUM

SCREENING TESTS FOR THE DETECTION OF ABNORMAL MILK

PHS Publication #1306

(X = DEVIATION)

LABORATORY APPARATUS

1. Tubes, plastic, clear, with vent, correct size (12.1 x 130 mm I.D.) .....
   Replaced when worn .....
   Held firmly in rack (may be cemented in place) .....
   Vents positioned to avoid spilling .....
2. Caps, calibrated, metal, 25 gauge (B&S) with 1.09-1.10mm orifice in center .....
3. Racks, aluminum, specially designed for WMT .....
   Hold 10 tubes tightly .....
4. Measuring square to read directly in WMTL/ml .....
   Correctly calibrated .....
5. Clock, preferably 8 inch wall clock with sweep second hand .....
   Second hand extends over marks on circular dial .....
6. Cornwall pipetting outfit (Becton-Dickinson No. 1251) 2 ml size .....
   Attached to 14 gauge cannula (B-D No. 1250 NR) of correct length .....
   Adjusted to deliver 1 ml of reagent (accuracy checked).. _____
7. Automatic pipette,
   Assembled from B-D No. 1250 S (syringe) and B-D No. 1250 MH (metal holder) .....
   Properly assembled with cannula .....
   Adjusted to deliver 2 ml of sample (accuracy checked)... _____

REAGENT

Equivalent to reference standard (Norden's Mastest Soln. Control P. 728) .....
Correctly diluted .....
With sterile buffered dilution water (See Standard Methods).. _____
Diluted solution not over 48 hours old .....

SAMPLES

Samples cooled immediately and maintained at 32°-40° F... _____
Temperature recorded upon receipt at laboratory..... _____
Temperature determined by the thermometer in pilot bottle .. _____
Held at 32° - 40° F at laboratory until tested (avoid freezing) .....
Preferably tested on day of collection..... _____
Well mixed immediately before test portion removed..... _____

PROCEDURE

a. Tubes rinsed in water before using..... _____
   Inverted and shaken to remove excess water..... _____
   Two ml of sample pipetted into tubes..... _____
   Foam avoided by discharging alongside tube..... _____
   Automatic pipette rinsed in water ..... _____
   And in sample before pipetting ..... _____

b. Caps arranged right side up in front of rack ..... _____

c. Reagent warmed to about 35°C (to make milk-reagent mixture 24° ± 2°C)..... _____
   Recirculated to bring warm reagent into pipetting outfit ..... _____
   Added rapidly to milk in two 1 ml volumes ..... _____
   Delivered ¼" below surface of sample to avoid foam (side arm of pipetting outfit rests on top of tube) .. _____
   Tubes capped promptly with snugly fitting caps..... _____

d. Mixing step started about 30 seconds after adding reagent to first tube..... _____
   Tubes tilted forward 10 times and backward 10 times in a nearly horizontal position..... _____
   During forward tilt the more fluid samples in rack cover about ½ of cap ..... _____
   During backward tilt butts of tubes move down in an arc of about ¾" from horizontal ..... _____
   Mixing step completed in 8-10 seconds ..... _____

e. Tubes inverted within ½ minute after mixing..... _____
   Rack held in horizontal position awaiting starting time ..... _____
   Held vertically for 15 seconds to permit outflow ..... _____
   Caps removed and washed promptly after inverting ... _____
   Tubes allowed to stand at least one minute to permit drain down..... _____
   Clock located close to analyst at eye level or below.. _____

f. Results read in WMTL/ml (read to the top of the meniscus)..... _____
   Reported in 2 significant figures..... _____
   Any deviations in sample care or age recorded on report ..... _____

g. Tubes rinsed 3 times in warm (not hot) tap water..... _____
   Rack inverted and shaken to remove excess water.... _____

Prepared by- D. I. THOMPSON
Wisconsin State Laboratory of Hygiene
JULY 1, 1966

GPO 911-888

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THE USE OF SCREENING PROCEDURES IN THE DETECTION OF ABNORMAL MILK

Introduction

The milk regulations in most progressive areas require that milk shall be from one or more healthy cows; that it shall be practically free of colostrum; and that milk otherwise abnormal should not be offered for sale. This includes milk from cows with mastitis; cows treated with medicinal agents which may be secreted in the milk; and milk containing pesticides, herbicides, or other agents which are judged to be prejudicial to the well being of the milk consumer.

*Current Concepts of Bovine Mastitis** describes the normal milk concentration of leucocytes as being 100,000 per ml., and further considers a leucocyte count in excess of 500,000 per ml. as being indicative of mastitis or other abnormality. Cows in very early or very late lactation are likely to produce milk having excessive leucocyte counts by these standards whenever a low-grade or latent inflammatory condition is pre-existent.

What are leucocytes? They are a normal constituent of the blood and are commonly called white blood cells. The white blood cells, particularly the polymorphonuclear neutrophils, are one of the body's important defenses against infection. When an infection occurs in the body, such as mastitis, these cells migrate to the area in large numbers in an attempt to limit and combat the infection. In the event of an injury to the udder, these cells migrate to the area and help to restore the tissue to its normal condition.

The screening tests described in this booklet are all based on the number of leucocytes in the milk. They are only presumptive screening tests and are not intended for diagnosing mastitis. The screening tests will usually indicate whether the milk is within normal leucocyte limits. Again, a high leucocyte count may indicate mastitis, udder injury, colostrum milk, stripper milk, or, possibly, a disease condition at another location in the cow's body. Veterinary consultation should be sought for mastitis diagnostic purposes.

The screening tests presented in this booklet are four which are currently in common use, as well as a recent modification of the CMT

*National Mastitis Council, Inc., 118 West 1st Street, Hinsdale, Ill., 60521 (Price—$1.00).

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test. Each chapter has been prepared by a person with wide experience with that particular test. The Milk Quality Test (MQT)* has not been included in this publication because of the scarcity of well-documented, scientific reports concerning the significance of positive results in terms of leucocyte counts. For practical purposes, the procedure appears to be similar to the California Mastitis Test, but the tests should not be used interchangeably in the absence of acceptable scientific evidence of their likeness.

There is no attempt made in this booklet to describe the various environmental, management, mechanical, and sanitation factors which contribute to the spread of udder infections. The reader is referred to the abbreviated list of related reading material at the back of the booklet for information about the causes and control of mastitis. Your State land-grant college is an excellent source of information on this subject.

*Dairy Equipment Co., Madison, Wis., 53701.

THE CALIFORNIA MASTITIS TEST

D. E. Jasper, D.V.M., Ph. D*

I. History

Routine application of the Whiteside Test to milk from individual mammary quarters as well as to "bucket" milk samples in conjunction with Dairy Herd Improvement butterfat tests revealed a high level of abnormal mammary glands in commercial herds. Examination of representative samples by bacteriologic methods indicated that a significant number of milks having high cell counts failed to support growth of recognized mastitis pathogens. A method to demonstrate abnormal milk at the side of the cow was needed in order to convince the dairyman of the true incidence of irritated mammary glands in his herd.

A modification of the Whiteside Test conducted in glass tubes or vials was first developed and designated the "Field Whiteside Test." This test had the disadvantage of requiring a vial for each milk sample to be tested, often meaning that several hundred vials had to be prepared in advance with Whiteside Test reagent and that the cleaning of the tubes later presented an additional labor problem. The next step was to attempt to conduct a Whiteside Test with dilute sodium hydroxide in equal quantity to milk drawn into black cups directly from the udder. This had possibilities, but the reaction needed an activator to improve the possibility of applying a score that would correlate with cell numbers in the milk. It was found that anionic surface-acting chemicals, such as alkyl aryl sulfonate, improved that test, and finally it was learned that the latter provided an improved test without the sodium hydroxide. The neutrality of the alkyl aryl sulfonate made it possible to add a color indicator, namely bromcresol purple, thus making detection of acid and alkaline milks in addition to cells a distinct possibility.

The test ultimately developed was entirely different than any mastitis detection method previously employed. It was conducted in a white plastic paddle with four shallow cups for collecting milk directly and separately from the four mammary quarters at the side of the cow. The reagent was added in equal quantity by estimation from a squeeze bottle, and the reaction was scored within 10 seconds while mixing the reagent and milk by causing the fluid to swirl in

*Professor of Clinical Pathology, School of Veterinary Medicine, University of California, Davis, Calif.

the cups. In order to indicate the new and unique nature of the test, and to designate clearly its origin, it was designated the California Mastitis Test or CMT.

II. Advantages

The advantages of the CMT may be conveniently divided into two categories listed in detail under A and B:

A. Simplicity and Accuracy of the Test

1. The reaction is with deoxyribonucleic acid (DNA) of the cell nucleic. Therefore, it is a measure of the concentration of entire cells entering the milk. It is truly what it is claimed to be, namely, an indirect measure of cell concentration in milk. The Whiteside Test comes closest to CMT in nature of the reaction, but fat content of the milk is also involved in the visible Whiteside reaction. The catalase test is not necessarily specific for whole or intact cells; and, therefore, positive reactions do not always correlate with cell numbers in the milk.

2. CMT is a sensitive test and, for this reason, while developed for quarter sample milk, it may be applied to "bucket" and "tank" milk with equal accuracy.

3. CMT can be graded with reasonable accuracy into five different scores. The cell count ranges for each of these scores has been clearly demonstrated.

4. The test reaction has a good record of repeatability on the same milk sample.

5. Correlation of CMT results with reliable leucocyte counts is equal to or better than is the case with other screening tests.

6. A CMT score is more accurate than isolated single cell counts.

7. Methods are available whereby scoring of CMT can be standardized among different people.

8. Accidental presence of extraneous material (hair, straw particles, etc.) does not interfere with the reaction.

B. Cost and Convenience

1. The CMT reagent may be purchased in concentrate form* or diluted and ready for use.** One gallon of the diluted reagent will conduct 1,800 individual tests, bringing the cost per test to less than ½ cent.

2. Only simple, inexpensive equipment is needed. For cowside, bucket milk, and occasional tank samples, only a plastic paddle and squeeze bottle for reagent are required. For routine laboratory screening, automatic syringes to deliver 2 ml. of milk and reagent, respectively, are desirable. Precise volumetric equipment is not needed.

*Dairy Research Products, Inc., Fairfield, Maine.
**NASCO, Fort Atkinson, Wis.

3. The test is conducted very simply and very rapidly. A great many tests can be completed per day by a single operator.

4. No labor is required for preparation or clean-up, even after hundreds of tests have been made. The CMT reagent has a cleansing action; thus, merely rinsing the plastic paddle in clear water prepares it for the next test.

5. The ease and rapidity of the test render repetition of the test very practical whenever doubtful reactions are encountered, or in the event that double screening is desired upon any category of samples.

6. Special environmental conditions are not required. The test can be made on milk at refrigerator temperature or at any reasonable room temperature.

7. If desirable, certain end-points can be selected; i.e., 1 million leucocytes, and milks screened only for reactions that score either higher or lower.

III. Disadvantages

1. Scoring of the test is somewhat subjective, allowing possible variation between testers or by the same tester upon duplicate tests. This is also true of the Modified Whiteside Test.

2. Test scores represent a range of leucocyte content rather than a precise number—a characteristic which is also true of the Modified Whiteside, Catalase, and Wisconsin Mastitis Tests.

3. Slides for the direct microscopic count can be filed for a permanent record. With the CMT, only the results can be recorded and kept on file, but this is also a disadvantage of the other tests.

4. The milk must be reasonably fresh, not over 2 days old, when kept under refrigeration. Bacterial action and preservatives destroy the reactivity of DNA with the reagent. Other tests are also subject to change in reaction with older milk.

IV. Equipment and Materials Needed

A. For cowside, bucket, and occasional tank testing:

1. CMT reagent.
2. Polyethylene squeeze bottle and special plastic paddle.

B. For programed screening of tank samples, it is faster and possibly more accurate to dispense milk and reagent with automatic syringes which can be set to deliver 2 ml. A suitable equipment list includes the following:

1. One Cornwall continuous pipetting outfit.
2. One Cornwall 2 ml. syringe.
3. One Cornwall metal pipetting holder.
4. One Cornwall 4-inch laboratory cannula.

A sink and cold water faucet, or a bucket for waste and rinse water, complete the list of equipment and materials needed.

V. The Conduct of the CMT

The CMT was designed originally to be made at the side of the cow. To keep the individual quarter milk samples separate, a white plastic paddle was designed having four shallow cups marked A, B, C, and D. When bucket or tank samples are tested, the four cups are used for four different samples.

It is recommended that 2 ml. of milk, or the amount that will remain in the cup when the paddle is tipped to a near vertical position, be used for each test. To this is added an equal amount of CMT reagent. Too little or too much reagent may give a false result. It is especially important that the amount of milk not exceed the amount of reagent. Gently rotate the paddle in a circular pattern so that the milk and reagent thoroughly mix and swirl. This requires about 10 seconds at which time the mixture is scored while the paddle is in motion. When scoring is completed, rinse the paddle in clean water and shake off excess moisture. The paddle is again ready for use.

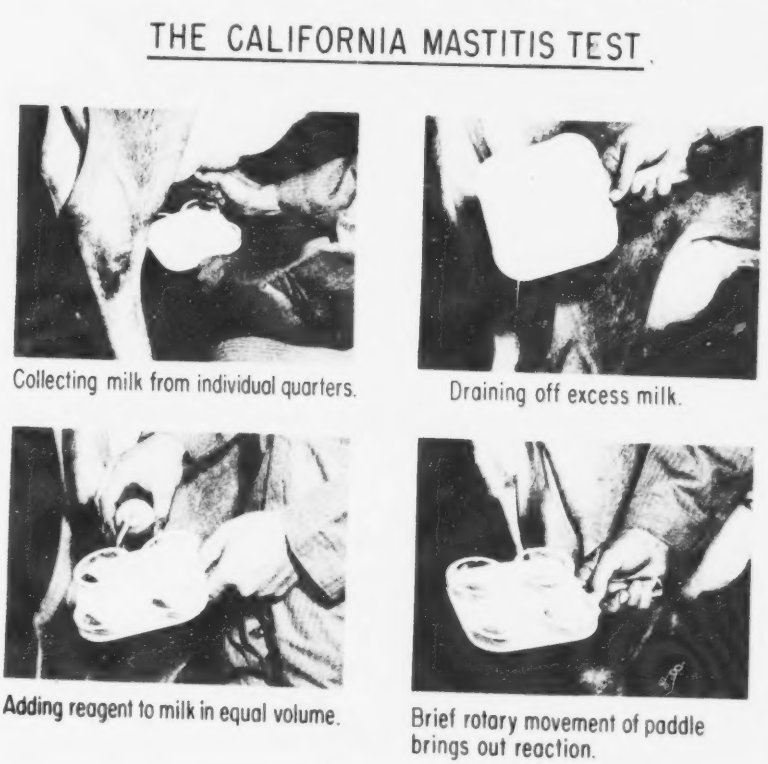
THE CALIFORNIA MASTITIS TEST
Collecting milk from individual quarters.
Draining off excess milk.
Adding reagent to milk in equal volume.
Brief rotary movement of paddle brings out reaction.

FIGURE 1.—The California Mastitis Test as performed at the side of the cow.

VI. Scoring and Interpretation of the CMT

The cell count range for the various reaction scores was originally published in 1956. Subsequent extensive experience has shown that these ranges as originally published are valid. A description of the visible reactions and their interpretations are given in the following table:

Grading and Interpretation

Symbol	*Suggested meaning*	*Description of visible reaction*	*Interpretation*
–	Negative----	Mixture remains liquid but no evidence of formation of a precipitate.	0–200,000 cells/ml. 0–25 percent PMN.
T	Trace-------	A slight precipitate forms and is seen to best advantage by tipping the paddle back and forth and observing the mixture as it flows over the bottom of the cup. Trace reactions tend to disappear with continued movement of the fluid.	150,000–500,000 cells/ml. 30–40 percent PMN.
1	Weak positive.	A distinct precipitate but no tendency toward gel formation. With some milks, the reaction is reversible; with continued movement of the paddle, the precipitate may disappear.	400,000–1,500,000 cells/ml. 40–60 percent PMN.
2	Distinct positive.	The mixture thickens immediately with some suggestion of gel formation. As the mixture is swirled, it tends to move in toward the center, leaving the bottom of the outer edge of the cup exposed. When the motion is stopped, the mixture levels out and covers the bottom of the cup.	800,000–5,000,000 cells/ml. 60–70 percent PMN.
3	Strong positive.	A gel is formed which causes the surface of the mixture to become convex. Usually there is a central peak which remains projecting above the main mass after the motion of the paddle has been stopped. Viscosity is greatly increased so that there is a tendency for the mass to adhere to the bottom of the cup.	Cell number generally over 5,000,000 /ml.

Grading and Interpretation—Continued

Symbol	*Suggested meaning*	*Description of visible reaction*	*Interpretation*
+	Alkaline milk.	This symbol should be added to the CMT score whenever the reaction is distinctly alkaline, as indicated by a contrasting deeper purple color.	An alkaline reaction reflects depression of secretory activity. This may occur either as a result of inflammation or in drying off of the gland.
y	Acid milk-----	Bromcresol purple is distinctly yellow at pH 5.2. This symbol should be added to the score when the mixture is yellow.	Distinctly acid milk in the udder is rare. When encountered, it indicates fermentation of lactose by bacteria.

In general, a score of 1 should be considered as indicative of 500,000 or more cells; and a score of 2 as evidence of 1,000,000 or more cells.

VII. Precautions

1. DNA in cells deteriorates upon standing. If milk is kept refrigerated, reliable readings can be obtained up to 48 hours. Thereafter, milk tends to lose its reactivity. Unrefrigerated milk cannot be tested accurately after about 12 hours.

2. Leucocytes tend to migrate with milk fat. Therefore, thorough mixing of milk just prior to testing is essential to assure a representative sample.

3. The CMT reaction must be scored between 10 and 15 seconds after mixing starts. Weaker reactions fade thereafter.

4. Because of the critical time factor in reading, use of boards or other devices containing more than four cups is not recommended. It is not possible to make accurate readings on more than four cups within a 5-second period.

5. It is helpful if testers can be trained using a series of milks of known count prepared by dilution of milk having a high cell content. This method promotes uniformity of results and confidence among testers.

6. In order to assure continued uniform performance and to safeguard against tendencies to drift in scoring habits, it is advisable for testers to be checked at intervals using diluted milks of known leucocyte content.

7. It is important to use CMT reagent produced under license. Only in that way can a standardized reagent be assured. Many nonstandard solutions have been proposed with inadequate evidence of standardized results.

8. Adequate lighting, as in other laboratory procedures, is important.

THE CATALASE TEST

C. W. Burch, B.S., D.V.M.*

I. History

This is a test to determine the amount of the enzyme catalase in plant or animal tissue or fluid. As a screening test in a quality milk program, it will detect abnormal milk which is of high cell content. These are usually leucocytes but may be epithelial cells sloughed from the udder during the "drying-up" period. Colostral milk also contains many of these epithelial cells, plus serum proteins, all of which increase catalase activity. There may be varying amounts of free catalase in milk from dead or disintegrated leucocytes and other body cells. Some bacteria, such as Staphylococci, and red blood cells also contain catalase.

II. Equipment

1. Test tubes, 16 ml. (16 x 125 mm. screw type) with ⅛ inch hole drilled in cap.
2. Test tube rack for 20 tubes.
3. One 10-cc. quick-delivery pipette.
4. One 1-cc. pipette (for H_2O_2) *or* one B–D Cornwall continuous pipetting syringe, 2 ml. capacity set to deliver 1 cc., with 2-inch straight cannula.
5. Aspirator bottle (4 liter) with tubing outlet, rubber tubing, and Mohr pinchcock for delivering distilled water.
6. Distilled water.
7. Three percent (3%) hydrogen peroxide. May use commercial, stabilized 3 percent H_2O_2, or make up from 30 percent stock solution (1 part 30% H_2O_2 to 9 parts distilled water). The hydrogen peroxide should be kept under refrigeration in tightly capped, dark bottles. The analyst should titrate the H_2O_2 solution at regular intervals to insure correct strength.
8. Ruler for measuring oxygen. Three mm. hard clear plastic is very satisfactory material for ruler. Test tubes vary some, depending upon the manufacturer. Make a separate ruler for each manufacturer's tubes by:

   (1) Introduce 1 cc. water, place blank ruler on tube, and mark water level.

*Extension Veterinarian, University of Wisconsin, Madison, Wis.

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(2) Introduce 9 cc. more of water and mark top level on ruler.

(3) Measure the distance between these two marks, divide into nine equal parts, and mark the ruler 10, 20, 30, up to 100.

III. Procedure

1. Shake milk sample and introduce 10 ml. into a 16 ml. test tube (16 x 25 mm. screw-top tube).
2. Add 1 ml. of 3 percent hydrogen peroxide.
3. Fill tube to overflowing with distilled water.
4. Cap tightly and invert tube.
5. Keep tubes for 3 hours at room temperature—about 72° F.
6. Read test by measuring oxygen gas formed with calibrated ruler. Read catalase test results directly from the ruler.

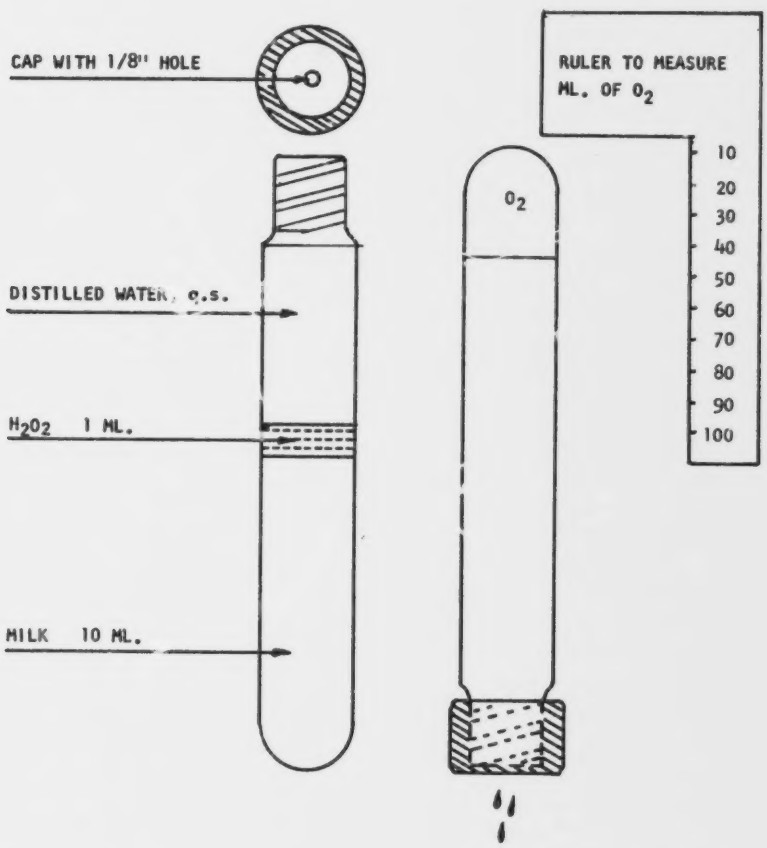

FIGURE 1.—Test tube with screw top and measuring device for use with the Catalase Test.

Chemical reaction involved in the catalase test:

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2 \uparrow$$

Relationship of Cells to Catalase Test Readings

We must relate all mastitis screening tests to something, and cell counts of milk seem the most logical. Some have questioned the catalase test because they could not always correlate the percentage of oxygen to the cell counts. We must remember that there can be widely varying amounts of free catalase in the milk sample for which there are no detectable cells.

The generally accepted comparison of catalase to the number of leucocytes is given in the following table:

Relation of Catalase Reactions to Leucocyte Counts

Catalase—percent oxygen	*Number of Leucocytes per cc.*
Less than 20	Less than 500,000.
20–30	500,000–1,000,000.
30–40	1,000,000–2,000,000.
Over 40	Over 2,000,000.

IV. Precautions

1. Keep all glassware chemically clean. This is a must to prevent the possibility of false positive reactions.
2. Do not pipette from or pour back into stock reagent (H_2O_2) bottle.
3. Remove liners from tube caps and smooth any rough areas around the bored hole.
4. Keep milk samples refrigerated at 40° F. from the time they are collected until tests are set up.
5. Shake milk samples well before testing.
6. Never test milk that has been frozen or overagitated (churned).

V. Summary and Conclusion

The catalase test is a good screening test to detect those producers who are selling milk that is abnormal because of excessive cell content. Since mastitis is the principal cause of high cell content, we consider the catalase test an excellent mastitis screening test, particularly for bulk milk samples. The greater the catalase content of milk, the greater the amount of oxygen produced in the test. The principal sources of catalase in milk are:

1. Leucocytes—due to inflammation of the udder.
2. Epithelial cells—sloughed from the udder during the "drying-up" period and the period just following parturition.

3. Excessive serum protein content of colostrum, which increases the catalase activity.

4. Free catalase—from dead or disintegrated leucocytes and other body cells.

5. Blood.

6. Bacteria, Staphylococci, and aerobic spore formers.

DIRECT MICROSCOPIC LEUCOCYTE COUNT TEST

A. Richard Brazis, B.S., M.S., Ph. D.*

I. History

The developers of the direct microscopic leucocyte count (DMLC), Prescott and Breed, reported the leucocyte counts of milk samples collected from 46 farms in New England in 1910. Although the counts ranged from 60,000 to 11 million per ml., the average was 1,500,000 which indicates that it is probable that mastitis was involved in many of the herds tested. Early investigators believed that a leucocyte count of 1,500,000 per ml. indicated mastitis; however, several recent investigators concluded counts exceeding 500,000/ml. are indicative of mastitis. Early studies by several investigators indicated that microscopic cell counts of milk collected directly from the cow's udder, rather than herd composites, were more desirable to demonstrate the presence of leucocytes and, in the case of bacterial mastitis, the causative organism. Evidence presented by many investigators has indicated that the foremilk should be used for slide examinations because it usually contains more organisms and leucocytes than milk drawn after several streams have been discarded; however, use of the strip-cup for the first two streams (in order to rinse out the teat canals) should still permit an adequate amount of foremilk to be sampled for examination.

II. Advantages

1. Rapid method which permits microscopic determination of leucocytes in 10–15 minutes for each sample examined.
2. Milk films can be prepared quickly, examined later, and kept for re-examination.
3. Cursory, tentative identification of bacterial flora may be made when examining milk film, although bacteria responsible for mastitis are not often observed in fresh, unincubated milk.

III. Disadvantages

1. Microscope is required.
2. Test cannot be conducted at side of cow.

*Senior Scientist, P.H.S., Milk Sanitation Research Unit, Milk and Food Research, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

3. Difficulties in measuring small (0.01 ml.) quantities. (Milk foam may cause inaccurate measurement with the 0.01 ml. pipette. The syringe may add metal filings as well as milk.)

4. Incorrect preparation and staining of slides. (If the film is not properly dried, the film will fall apart. During the staining of slides, enough time should be allowed for correct staining of cells.)

5. The small amount of milk examined.

6. Uneven spread over 1 square centimeter of slide; high or low cell counts may be made.

7. Failure to count the required number of fields. (Table 1 indicates the correct number of fields to be counted. If too few are counted, inaccurate counts may be made.)

8. Mistakes in observation and calculation. (Difficulty in seeing the difference between body cells and dirt as well as errors in arithmetic.)

9. Fatigue. (After the analyst examines several films, his eyes may become blurred.)

10. Initial cost of equipment is relatively high. (See section IV.)

IV. Equipment and Materials Needed

1. Sample containers.

2. Ice bath or preservative.

3. Level table in well-lighted room, reasonably free from dust and drafts.

4. Clean microscope slides, preferably with circular areas of 1 square centimeter each.

5. If slides are not available as in "4," use a guide plate, 2 inches wide and 4.5 inches long, having 16 or 24 round or square 1 sq. cm. areas.

6. Transfer instruments for 0.01 ml. quantities.

   a. Metal syringe with predetermined adjustment accuracy.

   b. Pipette—calibrated according to APHA specifications to deliver 0.01 ml. of milk.

7. Bent point needle—for spreading milk from pipette over an area of 1 square centimeter.

8. Drying surface—dust-free level surface with suitable heat source for drying films at 40°–45° C.

9. Forceps—for dipping and holding slides.

10. Trays or jars equipped with tight-fitting covers for holding solvents and stains and/or for submerging slides.

11. Slide storage box—for keeping slides free from dust prior to examination.

12. Compound microscope.

   a. Binocular preferred.

b. 1.8 mm. oil immersion objective, as well as low and high power objectives.

c. Substage moved by rack and pinion carrying an Abbe condenser with iris diaphragm.

d. Mechanical stage.

e. Oculars—5× or 10×.

f. Microscope lamp.

g. Stage micrometer slide—ruled in 0.1 and 0.01 mm.

h. Hand tally.

13. Methylene blue chloride, certified dry stain or Levowitz-Weber modification of Newman-Lampert prepared staining solution.

14. Immersion oil—refractive index 1.51-1.52 at 20° C.

15. Ethyl alcohol, xylene, tetrachlorethane, glacial acetic acid, fine filter paper (Whatman No. 42 or equivalent).

V. Conducting the Test

Adjustment of the microscope and calculation of the microscopic factor are necessary prior to the examination of stained milk films. These procedures are indicated on pages 94 and 95 in the 11th edition of *Standard Methods for the Examination of Dairy Products* (1960).

After cleaning the slide, shake sample vigorously 25 times. Using the 0.01 ml. pipette or mechanical syringe, dip either into milk (not foam layer) and rinse by drawing in and expelling portions several times. Withdraw test portion with tip below surface of milk and remove excess milk from exterior of tip of pipette or syringe using clean paper tissue or cloth. Transfer sampled milk to slide and expel the 0.01 ml. portion while holding either instrument in a vertical position. Using the bent needle or syringe extended piston tip, spread test portion evenly over an exact square centimeter area. Wipe needle and rinse pipette or syringe in clean water 25°–35° C. several times before use and between samples. (Although an 0.01 ml. transfer loop can be used instead of the syringe or pipette for obtaining portions of samples, it is not as accurate and will result in additional errors.) Legibly (and if necessary, indelibly) identify each film and each slide of films prepared.

After spreading films, fix them by drying at 40° C. within 5 minutes on a level surface reasonably free from dust, insects, and drafts. (Caution: Do not heat film too rapidly or film will crack and/or peel from the slide during later treatments.) In order to avoid evaporation and/or water absorption (causing changes in reagent and stain concentrations), as well as contamination of solutions, store "in-use" solutions in tightly sealed containers when not in use.

One of several methods recommended for leucocyte examinations utilizes the Levowitz-Weber modification of the Newman-Lampert stain. This stain is prepared by adding 0.6 gram of certified methyl-

ene blue chloride slowly to 32 ml. of 95 percent ethyl alcohol and 44 ml. of tetrachlorethane (technical) in a 200 ml. flask. Agitate to dissolve. Store at 44°–45° F. for 12 to 24 hours, then add 4 ml. glacial acetic acid. Filter solution through fine filter paper (Whatman No. 42 or equivalent) and store in tightly closed container with plastic closure. (Contamination of solution with water may cause difficulties with this stain.)

Slides having fixed dried milk films are submerged in a jar of staining solution for 2 minutes. Rest edge of slide on absorbent paper and drain off excess stain. Preferably use forced air to dry films thoroughly. Rinse slides in three changes of tap water at 100°–110° F., then drain and air dry films (without heat or drafts). Examine films microscopically.

The microscopic examination of stained milk films is done using the oil immersion objective. After placing the slide on the mechanical movable stage of the microscope, place one drop of immersion oil on each film. Preferably, count all leucocytes observed in every microscope field running completely across the film. (This procedure permits examination of a sufficient area of the film for reasonably reproducible results with less fatigue.) Because of uneven distribution of leucocytes and other body cells, especially in samples containing low numbers of leucocytes, actual estimates should be reported only after careful examination of not less than the minimal number of fields specified. (Where additional accuracy is desired, additional fields may be examined.) Alternatively, select successive rows at right angles to each other across film, starting about midway on any side and two to three fields in from edge of field. If it is necessary to examine 30 fields, select additional rows about 2 mm. apart, and continue to count the number of fields required based on the leucocyte density of the sample. The number of fields to be examined, by either method, can be determined by reference to Table 1.

TABLE 1.—*Determination of number of fields to be examined during the direct microscopic examination of leucocytes*

	Number of Fields to be Examined			
Range of leucocytes (Cts./ml.):				
30,000–300,000	30	40	50	60
300,000–3,000,000	20	20	20	30
Over 3,000,000	10	10	10	20
Microscopic factors	300, 000	400, 000	500, 000	600, 000
Field diameter (mm.)	0. 206	0. 178	0. 160	0. 146
Satisfactory eye oculars with 1.8 mm. oil immersion objective.	5X (6X or 6.4X)	5X (6X, 6.4X or 7.5X)	10X (7.5X or 8X)	10X (12X or 12.5X)

If the analyst is using a microscope equipped with 10X oculars and has predetermined the microscopic factor to be 500,000, Table 1 shows that the minimum number of fields to be examined (dependent on leucocyte density) is ten (10). After the examination of 10 fields, the analyst can divide the number of leucocytes observed in all fields by 10 and then multiply this by the predetermined microscopic factor; e.g., if 40 leucocytes were observed in 10 fields, dividing 40 by 10 and multiplying by 500,000, or $\frac{40}{10} \times 500{,}000$; this would be equivalent to 2 million leucocytes/ml. Since this count of leucocytes is not considered accurate when only 10 fields are examined, another 10 fields must be counted. If, after counting a total of 20 fields, a count of 70 leucocytes is obtained (40+30), the leucocyte count/ml. is determined by the formula:

$$\frac{\text{leucocytes counted in all fields}}{\text{numbers of fields examined}} \times \text{microscopic factor or}$$

$$\frac{70}{20} \times 500{,}000$$

which gives 1,750,000 leucocytes/ml. of sample.

Since, from Table 1, the examination of 20 fields is considered adequate for a leucocyte count of 1,750,000/ml. of milk with a microscopic factor of 500,000, the determination of the leucocyte density is completed for this sample.

After the slides have been examined, immersion oil on the films can be removed by dipping the slide in xylene for 15–20 seconds and allowing the films to air dry. Slides with films can be stored in slide boxes for future observations. (Care should be taken to avoid accumulation of pencil marks on slides, since their presence will interfere with staining procedures or future re-examination of films.)

VI. Interpretation of the Results

Leucocytes are normally found in milk, but, when present in excessive numbers (greater than 500,000/ml.) in milk directly from cow quarters, they may indicate physical injury, an infection, or milk which may have been collected from cows early or late in their lactation period. Milk sampled from old cows may show large numbers of body cells in addition to leucocytes.

Herd composite samples having leucocyte levels of 500,000 per ml., or greater, suggest mastitis; in which case, foremilk samples (taken after discarding first two streams of milk into strip cup) should be taken from individual quarters of each cow in the herd to determine the affected quarters. Where quarter samples exceed 1,000,000 leucocytes/ml., veterinary treatment is generally required.

VII. Precautions

On a routine basis, it is impractical to perform the leucocyte count on the milk from individual cows. It is generally more desirable to collect samples of herd composite milk samples and examine these samples microscopically. Direct microscopic leucocyte counts made on individual or herd composite milk samples may tend to be misleading due to improper preparation of milk film, faulty straining, or faulty counting procedures.

THE MODIFIED WHITESIDE TEST

H. C. Temple, D.V.M.*

I. History

The Modified Whiteside Test, as developed by Murphy and Hansen in 1941, was used to detect evidence of mastitis in a single quarter of a cow's udder. Reactions to the test were found to be closely related to congestion or inflammation in the gland tissue and the leucocyte concentration in the milk secretion. Murphy found that refrigeration of milk samples had a tendency to slightly intensify the reaction and that it was further intensified if a 2:5 ratio of reagent to milk was used instead of a 1:5 ratio as described in the original study. When these two intensifying factors were applied to individual quarter milk samples, it was found that too many false positive reactions occurred; so he advised that fresh quarter milk samples should be tested with a 2:5 ratio of reagent to milk and a 1:5 ratio used if the samples had been refrigerated before testing. In this way, comparable results were obtained on the same sample whether fresh or refrigerated.

In New York, it was found necessary to use both intensifying factors to make the test practical for use on the blended milk produced from entire herds. The milk is under refrigeration when available for testing and the 2:5 ratio of reagent to milk gives the increased sensitivity required. Modified Whiteside Tests made in this manner were generally negative on all bulk milk samples tested that contained 400,000 cells or less. Reactions start to take place at about 500,000, at which point many slight trace reactions can be expected. As the leucocyte count increases, the percentage of positive reactions increases and stronger reactions are observed. High cell count milk, one to several million, consistently produces reactions that are in direct relation to the leucocyte concentration.

The Modified Whiteside Test is quick, simple, and inexpensive. It can be made on the farm or in the milk plant laboratory. All of the equipment needed can be purchased in local stores at a very nominal cost; anyone can quickly learn the technique with proper instruction and practice. Experienced operators find that the reactions closely approximate the actual leucocyte counts of the milk.

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II. Advantages

1. Equipment and materials for running the test are inexpensive.
2. The test can be run on individual quarters, or bucket milk, or on tank milk.
3. Results of the test are rapidly available, and suspicious quarters may be examined immediately if test is run on the farm.

III. Disadvantages

1. Reading of test results may vary slightly between testers.
2. Tests should be completed within 2 days of sample pickup.
3. Tests cannot be stored for future reference as with DMLC.
4. Test results indicate a range of leucocytes rather than a specific number.

IV. Equipment and Materials Needed

1. *A smooth, dark surface for making the test.* Black bakelite sheets or ordinary window glass on a black background makes a suitable working area. Glass may be painted on one side for this purpose. Good-quality plate glass may not be suitable, because the highly polished surface may permit the milk to run over too large an area before the test can be completed. The size of the work area is dependent on the operator and the number of tests to be made at one time.

2. *Sodium hydroxide, NaOH, 4 percent solution.* The sodium hydroxide should be stored in flint glass or Pyrex containers in order to preserve the proper concentration. Ordinary glass will permit the solution to become cloudy and form a white precipitate in a relatively short period of time. If cloudiness develops or a precipitate forms, the solution should be discarded. The work-table supply may be stored in 2-ounce dropper bottle.

3. *Medicine droppers.* These should be of good quality and designed to deliver approximately the same amount of milk per drop as sodium hydroxide from the dropper bottle. Separate droppers must be used for the milk and the reagent.

4. *Applicator sticks.* These are slim, wooden sticks, 6 inches long, used for stirring the milk-sodium hydroxide mixture to bring out the reaction.

5. *Paper cups.* Two paper cups or small containers of 0.5-pint size of any kind are required for each operator. One is filled with clean water and the other used for the discharge when the milk dropper is rinsed between samples.

6. *Light.* Good light is essential for proper interpretation. Reflected light from a gooseneck-style lamp seems quite suitable, but it should not be kept close enough to the tests to warm the work area.

7. *Picture guide for interpretation.* This is quite essential for uniform results by the same operator or different operators. A photographic guide has been made available by the New York State Mastitis Control Program* (see fig. 1). This shows excellent detail of Modified Whiteside reactions on milk with various leucocyte counts.

V. Collection and Storage of Milk Samples

The Modified Whiteside Test can be made on the farm or in the milk plant laboratory from collected samples. Bulk tanks must be thoroughly agitated before sampling, and it is advisable to use a milk thief to remove the sample. Weigh-tank samples from can deliveries may be collected as soon as a producer's milk is dumped. An adequate sample should be collected, but the container should not be filled too full in order to permit thorough mixing later. All samples must be refrigerated from the time of collection, and the tests should be completed within 1 or 2 days.

VI. Conducting the Test

Before the Modified Whiteside Test is made, each individual sample should be thoroughly mixed, but violent shaking should be avoided. Sedimentation in the bottom and the cream layer at the top must be thoroughly incorporated throughout so that a representative sample can be removed. Five drops of milk are then placed on a black bakelite sheet or glass plate with a dark background. The dropper should be immediately rinsed with clean water a few times so that it will be ready to measure the next sample. It is not necessary to use sterile droppers. The milk should not be spread over too great an area, as proper mixing with the reagent may be difficult. Two drops of 4 percent sodium hydroxide are added, and the mixture is stirred very rapidly with an applicator stick held at a low angle for at least 20 to 25 seconds. The test mixture should be confined to a relatively small area during stirring in order to get the maximum "beating" action possible; but, when the test is almost completed, it should be gradually spread over a larger circular area slightly larger than a half dollar (3½ cm. diameter). Good light is essential for proper interpretation. The test should be completed on each individual milk sample before others are started.

VII. Grading Reactions

Negative (--). The mixture is opaque and milky in appearance and entirely free of precipitate throughout. The leucocyte count is usually under 0.5 million per ml.

*Available at $1.50 per copy from the photographer: C. Hadley Smith, the Carey Building, Ithaca, N.Y.

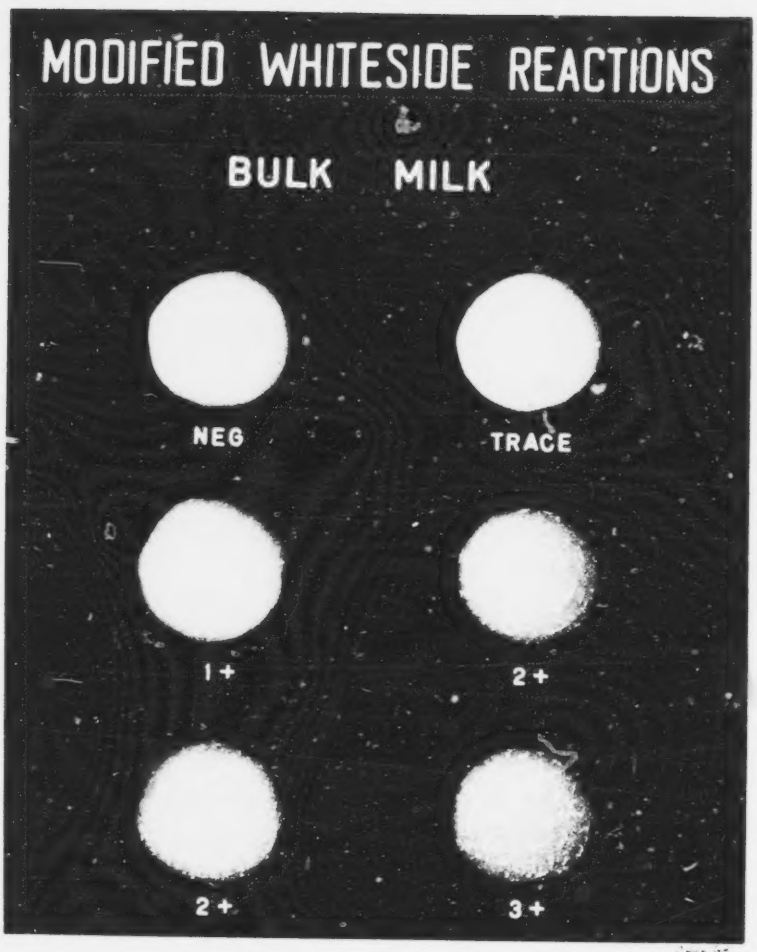

FIGURE 1.—Illustration of reactions to Modified Whiteside Test on bulk milk samples.

Trace (T). The mixture is still opaque and milky but with fine particles of coagulated material present. These may be few or many but show no tendency to clump. The leucocyte count is usually between 0.5 and 1.0 million, but a few may be slightly higher.

Positive (1+). The background is less opaque but still somewhat milky. Larger particles of coagulated material are present and thickly scattered throughout the area. Slight clumping will be observed. The leucocyte count is usually between 1 and 2 million with a few slightly higher.

Positive (2+). The background is slightly watery in appearance with large clumps of coagulated material. Fine strings or threads may form if stirring has been very rapid. The leucocyte count is usually 2 million or over and may be several million.

Positive (3+). The background is definitely watery and whey-like with large masses of coagulated material that may form into strings and threads. The leucocyte count is usually several million.

Milk with a high leucocyte count that produces strong reactions often thickens shortly after stirring is started and has a tendency to follow the applicator stick in a somewhat gummy mass.

VIII. Precautions

Variable results are sometimes obtained by different persons making the test on the same sample. If comparisons are to be made, they should take place at the same time and not one to several days later, after the samples have been contaminated from the first test and a possible bacterial breakdown of the constituents of the milk has taken place. Other possible reasons for variations are as follows:

1. The sample may not have been properly mixed by each person before making the test.
2. The droppers used for measuring the milk and reagent may deliver different quantities of material so that the result is not a true 2:5 ratio.
3. The milk may be spread over too great an area before reagent is added and stirring started.
4. The droppers may contain air below the column of milk or reagent so that the first drop of either may be largely air bubble, which disturbs the 2:5 ratio.
5. The different angles of stirring with the applicator stick may affect results. The stick should be held at a low angle in order to get complete mixing of milk and reagent.
6. The variable speeds of stirring and time of stirring will definitely affect the final result. The mixture must be stirred *very rapidly* for 20 to 25 seconds with complete mixing of milk and reagent or the maximum reaction will not appear.

7. If the work area is permitted to become too warm from the close proximity of electric bulbs, the reactions may be distorted.

8. The sodium hydroxide mixture must be a relatively accurate 4 percent concentration. Continued use of old solutions that have developed a cloudy appearance with a precipitate will very likely affect the accuracy of the test.

The Cause of Positive Reactions

Positive Modified Whiteside reactions are caused by excessive leucocyte concentrations in the milk tested. This condition occurs as a result of mastitis in dairy cows with the accompanying congestion and inflammation of the glandular tissue. The positive reaction may be confirmed by bacteriological examination of quarter milk samples. A New York study indicating the correlation between Modified Whiteside reactions and two types of udder pathogens is illustrated in the table below.

Relationship Between Modified Whiteside Reaction on Bulk Milk Samples and Incidence of Some Mastitis Pathogens:

Modified Whiteside reactions	*Number of herds*	*Abnormal secretion cows (Percent)*	*Percent of quarters infected*	
			S. Agalactiae	*Hemolytic staphylococcus*
Negative	159	6.7	2.2	5.6
Trace	457	8.6	3.4	7.6
1+	343	10.8	6.6	9.1
2+	82	17.4	13.3	10.7
3+	7	25.8	17.0	11.8

Summary

The Modified Whiteside Test can be successfully used as a milk quality test or a herd mastitis index. A minimum of equipment and material is required to make the test, and anyone can quickly learn the technique. Experienced operators find that the various reactions closely parallel the actual leucocyte counts of the milk tested. It has been found that positive reactions are in direct relation to the amount of mastitis found in dairy herds. Udder infections with mastitis pathogens, together with poor hygiene and herd management, are closely related to positive reactions. Judicious treatment of infections, together with improved hygiene and herd management, will result in improved milk quality and a reduction in mastitis losses.

THE WISCONSIN MASTITIS TEST

D. S. Postle, D.V.M.*

I. Brief History of Background of Test

The indirect screening tests, which estimate cell content of milk by permitting a measurement of viscosity resulting from the mixture of detergent and milk, have many advantages. This principle was first used in the California Mastitis Test (CMT). The Wisconsin Mastitis Test (WMT) was developed because a need existed for:

1. An objective test (results measured instead of being judged visually).
2. A test that could be standardized.
3. A test that could accurately estimate cell content in bulk milk samples while permitting the examination of large numbers of samples.

II. Advantages of This Test

The Wisconsin Mastitis Test permits an accurate, objective method of estimating the cell content of milk. This test is an extension of the CMT in that it combines detergent and milk and permits a measurement of the resulting viscosity. Advantages of this test are:

1. WMT permits the examination of a large number of samples per unit of time. There is no delay for results and retests may be made rapidly.
2. WMT uses CMT reagent, which is readily available, inexpensive, stable, and noncorrosive. The reagent needs no special handling.
3. WMT employs an objective method of measuring the degree of reaction. Slight reactions can be measured as easily and accurately as medium to strong reactions.
4. WMT accurately estimates cell content of bulk, bucket, or quarter milk samples.
5. WMT uses inexpensive, simple equipment.
6. Equipment cleans readily with flowing tap water.
7. The WMT readily lends itself to voluntary herd health improvement efforts by individual producers. For example, problem cows in the herd may be identified by testing bucket samples

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collected by the farmer and held for testing by the practicing veterinarian. The WMT may be performed readily in the milk house or in the laboratory. High leucocyte count quarters may then be identified and confirmed by the WMT or by use of a cow-side paddle test.

III. Disadvantages of This Test

1. WMT does not lend itself to cow-side use as readily as the paddle-type tests (CMT et al.).
2. WMT has no permanent reaction that can be examined or re-examined at leisure, as the dried and stained smears for the direct microscopic leucocyte count.
3. Milk should be refrigerated promptly and preferably tested on day of collection. Reaction diminishes slowly in storage at 32°–40° F.

IV. Equipment* and Materials Needed

1. Aluminum rack:
   a. Formed from 18 x 8 inch, 0.040-inch thick aluminum sheet.
   b. Rack supports 20 disposable test tubes in 2 rows of 10 each.
   c. Tubes are held in place in rack by friction.
2. Plastic test tubes (12.5 x 125 mm.), Flex-vials with matching caps (fig. 1).
   a. Tubes have breather hole 65 mm. from outside bottom burned into sides.
   b. Caps have standard 1.15 mm. diameter center hole to permit outflow.
3. Continuous pipetting devices, 2 ml., two each, and continuous pipetting device, 10 ml., one each (Cornwall).
4. Cannula, 4-inch, 14-gauge.
5. Cannula, 2-inch, 14-gauge.
6. Rule, calibrated to measure increments of 5 mm. from the inside bottom of the test tubes (fig. 1).
7. CMT reagent, diluted 1 : 1 with distilled water.
8. Timing device with sweep-second hand.

V. Conducting the Test

The test is conducted in the following manner:

1. Pipette 2 ml. of well-mixed milk from each sample into each respective tube from a 2-ml. automatic syringe (from a continuous pipetting device) fitted with a 14-gauge cannula 1 to 2 inches long (fig. 2). The syringe may be rinsed in the following milk sample before measuring from that sample.

*Available from: Z. D. Roundy, 5130 Pepin Place, Madison 5, Wis.

2. Reagent is then added to each tube from a 2-ml. continuous pipetting device fitted with a 3¼-inch, 14-gauge cannula (fig. 3). Reagent should be added *below the surface of the milk* in each tube to provide good initial mixing of sample and reagent and to prevent the formation of foam which interferes with reading the test. (Two 1-ml. deliveries of reagent provides more uniform mixing of milk and reagent.)

3. Each tube is capped, and the milk and reagent is mixed by gently rocking the rack back and forth 10 times in about 10 seconds while the tubes are in a nearly horizontal position (fig. 4). The mixing action permits the fluid in the tubes to flow forward to cap and return 10 times. (The tubes are placed in the rack in such a position that during the mixing process the breather holes are all facing up to prevent loss of any of the milk and reagent mixture.)

4. The tubes are allowed to stand for 20–30 seconds.

5. The rack is then inverted and held in a vertical position to permit outflow for 15 seconds (fig. 5). Accuracy in timing the period of inversion is important. At the end of the 15-second outflow period, the rack is returned to an upright position and set aside for 2 to 3 minutes to permit all fluid to drain from the tube walls.

6. The test is scored by measuring the remaining fluid column in each tube (fig. 6). Results are recorded in millimeters.

7. Tubes and caps are washed in flowing tap water in preparation for the next series of tests. Excess water may be removed by shaking the rack.

VI. Interpretation of the Results

The Wisconsin Mastitis Test correlates well with direct microscopic leucocyte counts (correlation coefficient 0.9) and has a high degree of reproducibility (coefficient of variability of 9 percent). The following table permits conversion of WMT readings into total cell counts. (A WMT reading of 20 could be used for screening out milk supplies with 500,000 or more cells/ml.)

WMT Value	*Total Cell Count**
5	75,000
10	190,000
15	350,000
20	570,000
25	830,000
30	1,200,000
>30	>1,500,000

*Determination made from regression curve published in *Journal of Milk and Food Technology*, Vol. 27, September 1964, pp. 271–275.

VII. Precautions

1. Fluid columns in tubes, for either calibration or milk testing, are measured from the inside bottom of tube to the top of the meniscus.

2. When washing tubes and caps, care should be taken to insure adequate washing of outflow holes in caps. Do not wash tubes or caps in hot water.

3. Mechanical pipetting of reagent with a cannula is essential to uniform mixing of sample and reagent.

4. The tilting action used for completing the mixing of milk and reagent should be positive but gentle. This mixing action should be started within 30 seconds of the time of adding reagent to the milk in the first tube in the rack. Avoid violent shaking, as this destroys part of the reaction. When mixing, prevent loss of fluid through either outflow holes in caps or breather holes in tube walls.

5. Action used for inverting and returning rack to upright position should be positive, rapid, and smooth. Avoid unsteady motion.

6. During outflow, the rack should be held so that the tubes remain vertical during the entire timing period. For greater precision, milk samples may be tested in duplicate and the readings averaged. Any sample giving results with more than a 3-mm. discrepancy between the two readings should be retested.

7. Samples should be cooled promptly after collection and held at 32°–40° F. until tested.

8. Tubes and caps should be calibrated before putting new equipment into use and periodically thereafter. To calibrate, pipette with a 10-ml. continuous pipetting device, 6 ml. of distilled water at room temperature into each of the tubes in a rack. Cap the tubes and invert the rack to permit water to flow through the cap holes for 5 seconds. Return the rack to an upright position and, with the calibrated measuring rule, determine the height of the remaining water column in each tube. Satisfactory tube and cap combinations will permit a 27 to 29 mm. column of water to remain after outflow. The water column is measured from the inside bottom of the tube to the top of the fluid meniscus. If the water column is less than 27 mm. or greater than 29 mm. in height in any tube, the cap used on this tube should be discarded.

9. Accuracy of estimating the cell content of milk is improved if the test is limited to 10 tubes per rack.

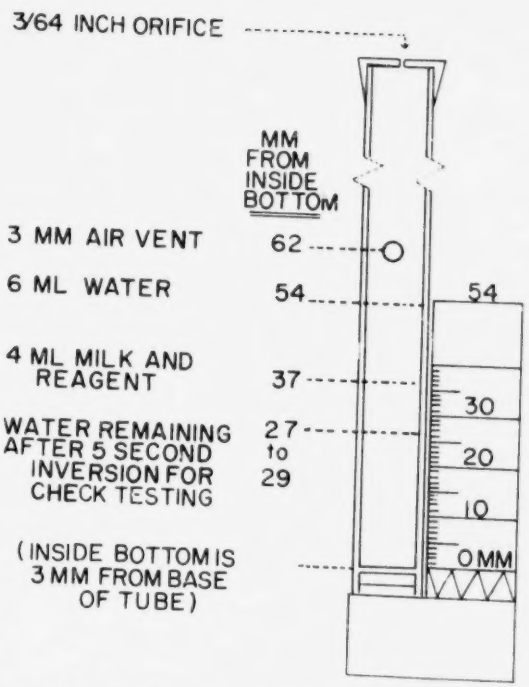

FIGURE 1.—Plastic test tube and ruler used with the Wisconsin Mastitis Test.

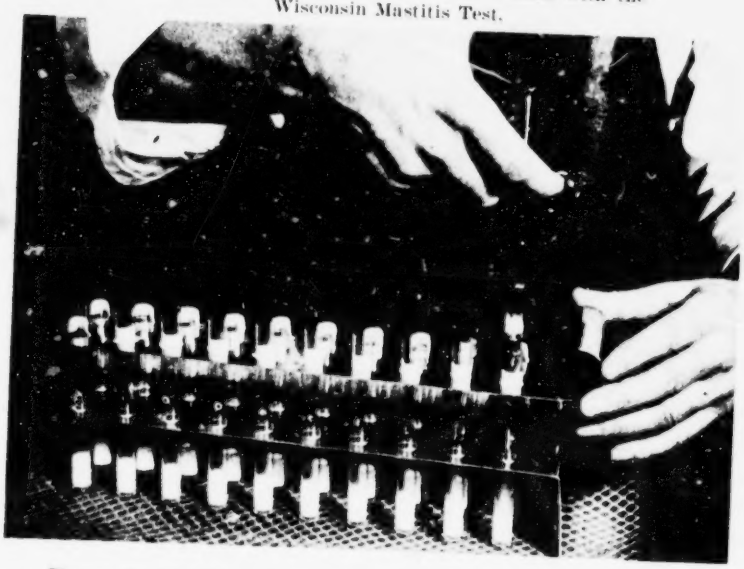

FIGURE 2.—Pipetting of milk sample in Wisconsin Mastitis Test.

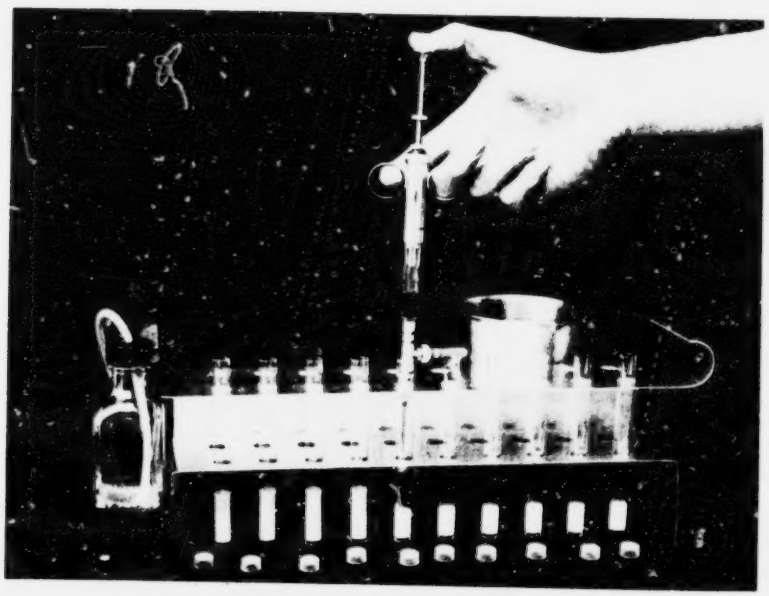

FIGURE 3.—Adding reagent to milk sample. Note that tip of cannula is below surface of the milk.

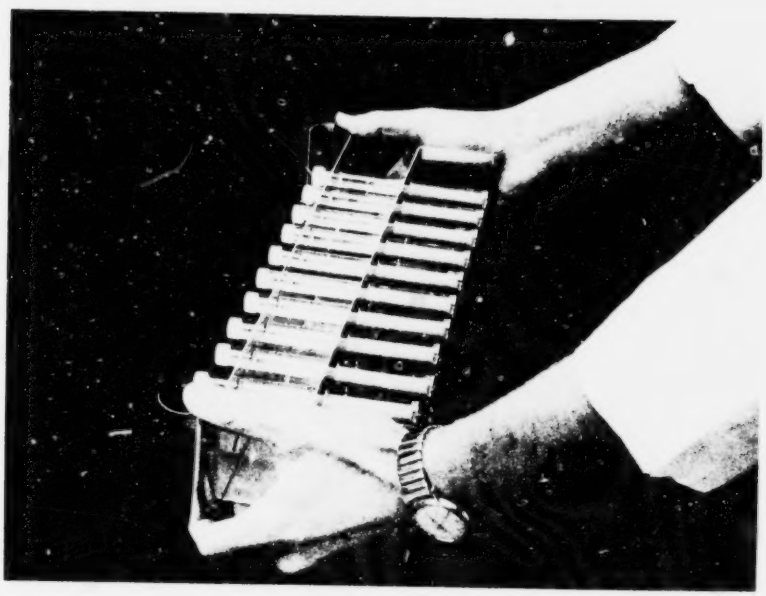

FIGURE 4.—Mixing reagent and milk.

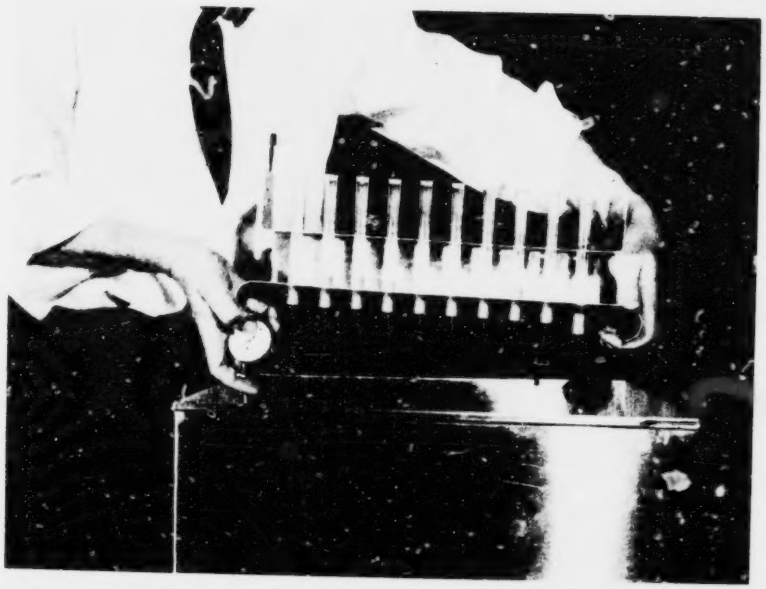

FIGURE 5.—Rack inverted and timed with a stop watch.

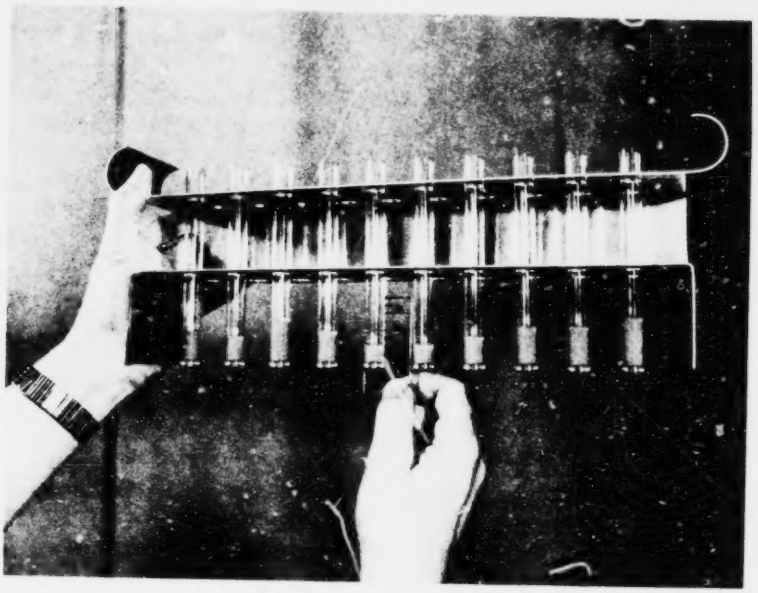

FIGURE 6.—Measuring remaining fluid in the test tube.

RELATED READING

"Current Concepts of Bovine Mastitis," National Mastitis Council, Inc., 118 West First Street, Hinsdale, Ill., 60521.

"Bovine Mastitis," Bulletin No. 525, Ontario Department of Agriculture, Parliament Buildings, Toronto, Canada.

"Milking Management and Its Relationship to Milk Quality," Agriculture Extension Service, University of California, Davis, Calif., 95616.

"Milking Machine and Mastitis," D. O. Noorlander, Dairy Equipment Co., Madison, Wis., 53701.

"The Modern Way to Efficient Milking," Milking Machine Manufacturers Council, F.I.E.I., 410 North Michigan Avenue, Chicago, Ill., 60611.

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